

Review

New Aspects of Physiological and Pathophysiological Functions of Adenosine A_{2A} Receptor in Basal Ganglia

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There is now growing interest in the functional role of adenosine A_{2A} receptors. Their distribution within the brain is restricted in the basal ganglia, particularly abundant in the striatum, which are thought to play a crucial role in the control of motor behavior. Indeed, newly developed A_{2A} receptor selective antagonists have a profound influence on motor functions, with anti-Parkinsonian activities in several animal models. Striatal spiny neurons serve as a major anatomical locus for the relay of cortical information flow through the basal ganglia. The GABA releasing projection neurons represent the A_{2A} receptor-mediated main target of adenosine. The GABAergic synaptic neurotransmission is regulated by adenosine via A_{2A} receptors on the presynaptic terminals. Blockade of this modulatory function by A_{2A} antagonists could repair striatopallidal abnormal neuronal activities provoked by striatal dopamine depletion in the Parkinsonian state. A_{2A} receptor antagonists provide a novel therapeutic potential for the treatment of Parkinson's disease.

Key words: adenosine; receptor; movement; basal ganglia; neuromodulation

Adenosine has a great variety of physiological effects on the nervous system and peripheral tissues. Drury and Szent-Györgyi¹⁾ first demonstrated the effects of adenosine on cardiovascular function in 1929. Subsequently, it became clear that adenosine, acting through specific receptors, was a potent biological mediator that modulates the activity of numerous cell types. These include various neuronal populations, platelets, neutrophils, and mast cells, and smooth muscle cells in bronchi and vasculature. In the brain, Kakiuchi *et al.*²⁾ first found to increase accumulation of adenosine 3',5'-cyclic monophosphate (cAMP) in brain slices upon electric stimulation, which was later shown to release adenosine,³⁾ in a manner blocked by methylxanthines.⁴⁾

Over the years, reports on possible functions for adenosine in the nervous system have increased steadily in number, with adenosine being implicated in epilepsy, cerebral ischemic preconditioning, sleep, and immune reactions within the brain.⁵⁾ Recent advances in molecular biology, biochemistry, cell biology, and behavioral pharmacology together with selective ligands for the various adenosine receptors have increased our understanding of the function of adenosine and its receptors. Four different subtypes of adenosine receptors are now identified and designated A₁, A_{2A}, A_{2B}, and A₃.^{6,7)} Each subtype couples with G-proteins, and has a distinct action on the cAMP regulatory system: the A₁ and A₃ receptors inhibit, but A₂ receptors stimulate adenylyl cyclase activity. Of the four adenosine receptor subtypes, A₁ receptors have been studied most extensively because pharmacological tools for the A₁ receptors have been available for almost 20 years and because of the widespread distribution of the receptors in the brain. Adenosine A₁ receptor activation has a depressant effect on neurons by reducing neurotransmitter release from presynaptic nerve terminals and increasing potassium conductance in postsynaptic cells.⁸⁾ In this respect, numerous reviews have focused on A₁ receptor ligands as potential therapeutic entities in various pathological states, e.g. hypoxia/ischemia, stroke, seizures, psychiatric disorders, nociception, and cognitive disorder.^{9-11,25)}

Due to the recent development of more selective tools for studying A_{2A} receptors, there is now growing interest in the functional role of these receptors. During a drug discovery program looking for adenosine receptors and their signal transduction, KF17837, (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine, and its 1,3-diethyl analogue, KW-6002, were found to be strong and selective antagonists for adenosine A_{2A} receptors.¹²⁻¹⁵⁾ In contrast to the widespread distribution of A₁ receptors within the CNS, A_{2A} receptors are localized in basal ganglia

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Abbreviations: SNr, substantia nigra pars reticulata; Gpi, the internal segment of globus pallidus; Gpe, the external segment of globus pallidus; STN, subthalamic nucleus; SNc, substantia nigra pars compacta; NMDA, N-methyl-D-aspartate; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Ach, Acetylcholine

and particularly abundant in the striatum* (i.e. caudate nucleus, putamen, and nucleus accumbens), the olfactory tubercle, and the external layer of the globus pallidus (GPe).^{14,16-23,67)} Since the basal ganglia are known to be a critical component of subcortical circuits involved in the integration of sensorimotor, associative, and limbic information to produce motor behavior, the selective distribution of A_{2A} receptors in the regions suggests a possible implication of these receptors in the control of these activities. In fact, the modulation of A_{2A} receptors by the A_{2A} antagonists strongly influences motor functions.^{24,25)} In particular, they had anti-Parkinsonian activities in several kinds of Parkinson's disease models in rodents and monkeys.²⁴⁻²⁹⁾

The aim of this article is to review first biochemical and pharmacological properties of the A_{2A} receptor antagonists, and then to provide information on new aspects of physiological and pathophysiological functions of the A_{2A} receptor in the basal ganglia. Finally, the therapeutic implications of A_{2A} antagonists will be discussed, focusing on Parkinson's disease.

1. Biochemical and Pharmacological Characterization of the A_{2A} receptor antagonist

1.1. Biochemical Properties^{13-15,30)}

The original adenosine receptor antagonists were xanthines such as caffeine and theophylline, which have little or no selectivity for this receptor. Subsequent modifications of the xanthine nucleus led to the development of antagonists with A_{2A} receptor selectivity, such as KF17837. The compound is a strong and selective A_{2A} receptor antagonist. KF17837 has a K_i of 1.0 ± 0.057 nM for the rat striatal A_{2A} receptor labeled with the A_{2A} receptor selective agonist [³H]2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS21680). KF17837 has 62-fold selectivity for the A_{2A} receptors versus rat forebrain A₁ receptors. The binding of KF17837 is reversible and of a competitive type. KF17837 at 10 μ M had no effect on adenosine transporter, on α_1 -, and α_2 -adrenoceptors, β -adrenoceptors, dopamine D₁/D₂ receptors, histamine H₁/H₂ receptors, muscarine M₁ receptors, nicotine receptors, and 5-HT₁/5HT₂ receptors, indicating high specificity.

In rat pheochromocytoma PC12 cells, KF17837 antagonizes cAMP accumulation induced by CGS21680, with an IC₅₀ of 53 ± 10 nM. No partial agonist activity is detected. cAMP accumulation by

the A_{2B} receptor activation is inhibited by KF17837, with an IC₅₀ of 1500 ± 290 nM.

KW-6002 has potency and selectivity for A_{2A} receptors similar to KF17837. The K_i is 2.2 ± 0.88 nM for rat striatal A_{2A} receptors and 68-fold selectivity for the A_{2A} receptors versus rat forebrain A₁ receptors.

More recently, a number of other A_{2A} receptor selective antagonists have been developed, and their properties reviewed.³¹⁾

1.2. Pharmacological properties - Action of the A_{2A} receptor antagonists in animal models of Parkinson's disease

Since one of the important functions of the striatum is motor coordination, a number of reports have focused on the role of the A_{2A} receptor in locomotor activities and behavior, using A₂ receptor agonists and antagonists.³²⁻³⁵⁾ However, because these earlier studies used non-selective adenosine receptor antagonists like theophylline or caffeine, the contribution of A_{2A} receptors to the motor responses was difficult to assess. Using KF17837 and KW-6002, it became possible to observe how the blockade of A_{2A} receptor affected motor response in different animal models. Thus these A_{2A} antagonists ameliorated motor deficits in several kinds of rodent behavior models.²⁴⁻²⁷⁾ In particular, KW-6002 could induce increased locomotor activity in reserpinized mice and reversed haloperidol-induced catalepsy in rodents.²⁵⁾

Unilateral injection of 6-hydroxydopamine preferentially destructs nigro-striatal dopaminergic neurons in the injection side. After the unilateral lesion of the nigro-striatal pathway, dopamine agonists cause vigorous turning behavior towards the contralateral side. This model is one of the most reliable rodent models for the assessment of anti-Parkinsonian efficacy. Indeed, KW-6002 turned out to potentiate the rotational response caused by a dopamine agonist or L-dopa.²⁷⁾

MPTP causes dopaminergic cell death with the onset of Parkinsonism in primates. This disease model is highly predictive of drug action in man. Orally administration of KW-6002 to MPTP-treated primates lead to a modest increase in locomotor activity coupled to a significant reversal of motor disability. The effect was not accompanied by abnormal movement such as stereotypy, or by nausea, vomiting, or other obvious peripheral side effects.^{28,29)} While these effects are not qualitatively different from those one might expect with a dopaminergic approach to Parkinson's disease, there is considerable interest in the failure of KW-6002 to provoke abnormal involuntary movements in MPTP-treated primates previously exposed to L-dopa, and which have dyskinesias in response to all dopaminergic drugs. Importantly, repeated administration of KW-6002 caused no tolerance to its anti-Parkinsonian effects.

* The primate striatum contains a structurally distinct caudate nucleus and putamen whereas these structures are not differentiated in the rodent brain and are collectively referred to as the neostriatum, corpus striatum, or striatum. The striatum of both primates and rodents contains a rostroventral extension, which is referred to as the nucleus accumbens or ventral striatum.

2. The basal ganglia and neuromodulator function of A_{2A} receptors

2.1. The current model of basal ganglia organization^{36,37)}

The basal ganglia are located in the telencephalon and consist of several interconnected nuclei: the striatum, globus pallidus external segment (GPe), globus pallidus internal segment (GPi), substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr), and subthalamic nucleus (STN) (Fig. 1).

The striatum is a major component of basal ganglia. The striatal neurons receive massive glutamatergic excitatory inputs (Fig. 1, corticostriatal pathway).

The major neuronal population in the striatum is represented by medium spiny neurons, accounting for almost 95% of all striatal neurons and using γ -amino-butyric acid (GABA) as a neurotransmitter. The remaining 5% of striatal cells consists of aspiny interneurons, including GABAergic and cholinergic interneurons. Medium spiny neurons are the only known projection neurons in the striatum and their outputs are directed to the major output nuclei of basal ganglia, i.e. GPi and SNr, via both direct and indirect pathways (Fig. 1). Neurons of the striatonigral direct pathway contain GABA plus substance P/dynorphin and directly project from the striatum to GPi/SNr. They provide a direct inhibito-

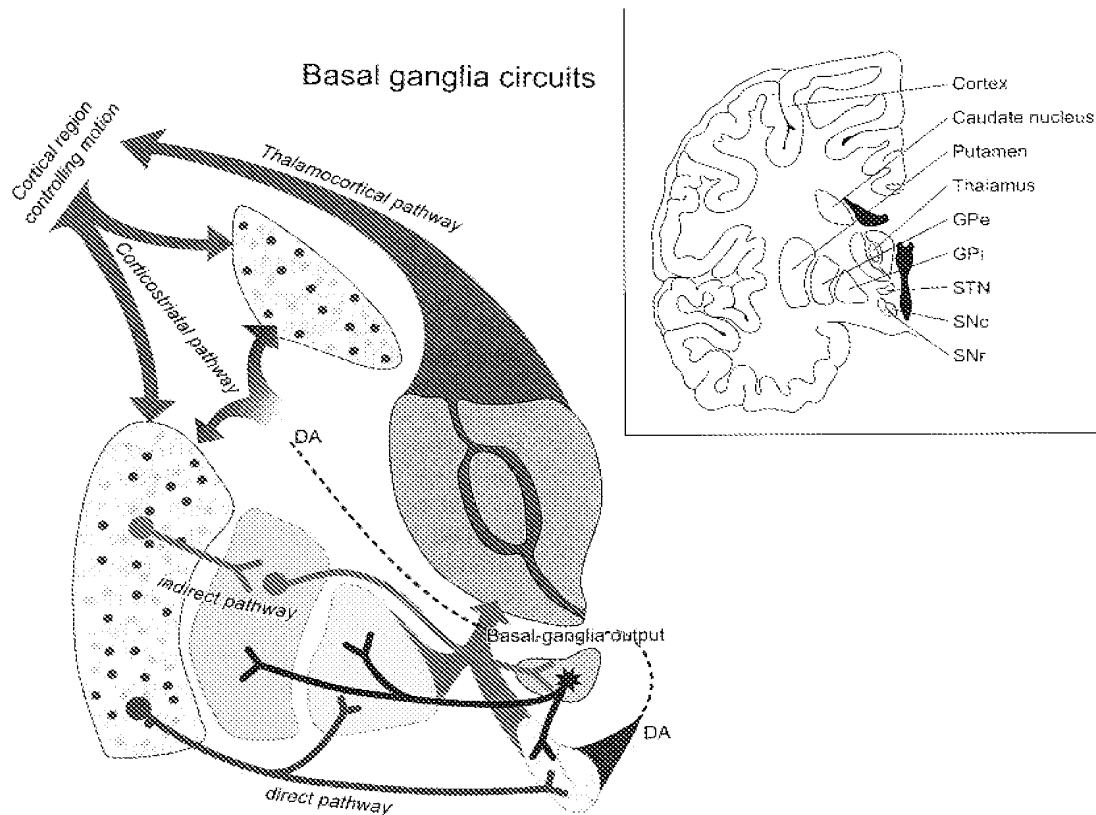


Fig. 1. Major Neuronal Circuits of the Basal Ganglia.

Inset, the basal ganglia consist of several interconnected nuclei: striatum (the caudate nucleus and putamen in primate), globus pallidus external segment (GPe), globus pallidus internal segment (GPi), substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr), and subthalamic nucleus (STN).

The basal ganglia circuits are functionally interposed between the cortex and the thalamus. The basal ganglia system constitutes a high degree of convergence and is organized as 'funnel' with the cortex as the entrance, the striatum as the intermediate, and the GPi/SNr acting as the output nuclei. The main task of the circuit is to process the signals that flow from the cortex, to produce an output signal that returns to the cortex through the thalamus, allowing the correct execution of voluntary movement. Excitatory projections are depicted as green and inhibitory projections are depicted as red or brown. Cerebral cortical neurons in layer 5 provide massive glutamatergic excitatory input to the striatum (corticostriatal pathway). The striatum comprises two populations of medium spiny projection neurons, which are scattered about in equal numbers throughout the striatum. Direct pathway neurons (red) contain GABA plus substance P or dynorphin and project directly to the output nuclei of basal ganglia, GPi/SNr. The indirect pathway neurons (yellow) contain GABA plus enkephalin and connect to the GPi/SNr via relays in the GPe and STN. From GPi and SNr, GABAergic output returns to cortex via the glutamatergic thalamic relay (thalamocortical pathway). Activation of the trisynaptic (Striatum-GPe-STN-GPi/SNr) indirect pathway results in disinhibition of GABAergic output neurons in the GPi/SNr, in opposition to the inhibitory influence of input from the direct pathway. Dopaminergic input (blue) into this synaptic network comes from neurons of the SNc that project to the striatum.

ry effect on GPi/SNr neurons. Striatal neurons in the striatopallidal indirect pathway contain GABA plus enkephalin and connect the striatum with the GPi/SNr via synaptic connections in the GPe and STN. This sequence of connection comprises, as follows (Fig. 1, also see Fig. 2-(a)). (1) an inhibitory GABAergic projection from the striatum to GPe; (2) an inhibitory GABAergic projection from the GPe to STN; (3) an excitatory glutamatergic projection from the STN to the GPi/SNr. Consequently, stimulation of neurons in the indirect pathway leads to inhibition of the GPe, disinhibition of the STN and excitation of the GPi/SNr. Thus, the opposing effects of inhibitory inputs from the direct pathway and excitatory input from the indirect pathway influence the output activity of the basal ganglia. GPi and SNr provide inhibitory inputs to thalamic nuclei, which project the excitatory neurons to the cortical region controlling motion (thalamocortical pathway). Increased activity of the direct pathway is associated with facilitation of movement and increased activity of the indirect pathway is associated with inhibition of movement.

Dopaminergic input into this synaptic network comes from neurons of the SNc that project to the striatum. In Parkinson's disease, degeneration of the dopaminergic neurons triggers a cascade of functional changes affecting the whole basal ganglia network. At striatal levels, dopamine appears to facilitate transmission along the direct pathway and inhibit transmission along the indirect pathway, these two opposite effects being mediated apparently by dopamine D₁ receptors on striatonigral neurons and D₂ receptors on striatopallidal neurons, respectively. Imbalance between the activity in the direct and indirect pathways and resulting alteration in GPi/SNr are thought to account for the hypo- and hyperkinetic features of basal ganglia disorders.

More recently, it has been recognized that adenosine appears to play an important role in functional response via the A_{2A} receptors in the striatum. Importantly, the A_{2A} receptors are specifically expressed on a subpopulation of medium spiny neurons in the indirect striatopallidal pathway but not in the direct striatonigral pathway.¹⁷⁾

2.2. Adenosine modulates the GABAergic circuit via A_{2A} receptors

Using electrophysiological techniques, intracellular recording and whole cell patch clamp recording, in rat striatal slices, we found an adenosine A_{2A} receptor-mediated disinhibition mechanism in medium spiny projection neurons.³⁸⁾ As a very useful tool to identify the A_{2A} receptor-mediated mechanism, the newly developed A_{2A} receptor selective antagonist KF17837 together with the selective agonist CGS21680 was used. Thus we demonstrated that the A_{2A} agonist suppressed inhibitory postsynaptic currents (IPSCs) evoked by stimulation within the striatum

in a manner selectively inhibited by the A_{2A} antagonist. This indicates that the A_{2A} receptors serve to suppress GABAergic synaptic transmission onto these striatal neurons. We further showed by analyzing miniature IPSCs* that this suppression of GABAergic synaptic transmission by adenosine was mediated by presynaptic, but not postsynaptic, A_{2A} receptors.³⁸⁾ Indeed, in the experiments with striatal synaptosomes, CGS21680 inhibit GABA release in a manner blocked by KF17837, indicating the suppression of GABA release by adenosine via the A_{2A} receptors on the striatal nerve terminals.⁶⁷⁾

These results implied that the A_{2A} receptor regulated the activities of striatal projection neurons by relieving GABA-mediated inhibition of medium spiny projection neurons.

In the striatum, the GABAergic input into GABA_A receptors on the spiny projection neurons might come from their axon collaterals (branches) and GABAergic interneurons.⁴⁰⁻⁴²⁾ The GABA release from the axon collaterals might produce mutual inhibition between spiny neurons to serve a feedback inhibition circuits within the striatum, while GABAergic inputs from the interneurons should serve a feedforward inhibition mechanism. Thus, activation of A_{2A} receptors on these striatal nerve terminals** by adenosine would relieve either or both of these inhibition mechanisms, resulting in increase of the activity of the projection neurons.

Using similar electrophysiological methods with the globus pallidus (GP) GABAergic projection neurons, we found that CGS21680 increased IPSCs evoked by stimulation within the GP in a manner blocked by KF17837 and that the increase was attributable to presynaptic, but not postsynaptic A_{2A} receptors. This indicates that, in the GP, adenosine presynaptically increases GABAergic synaptic transmission via the A_{2A} receptors.³⁹⁾

Pallidal GABAergic projection neurons receive massive GABAergic input from the striatal projection neurons as well as their axon collaterals.³⁶⁾ A_{2A} receptor proteins exist in the GP,²³⁾ but no mRNA has been detected in the GP neurons.^{18,20)} Hence A_{2A} receptors in the GP are most likely located on the axonal terminals of striatal projection neurons; therefore most of the increase of GABAergic transmission

* mIPSCs; spontaneous IPSCs in the presence of tetrodotoxin to block the propagation of action potentials to the terminals. mIPSCs are attributable to spontaneous release of GABA from the presynaptic terminals. The A_{2A} agonist and antagonist changed the frequency of mIPSC without affecting the amplitude, showing a presynaptic action of A_{2A} receptors to regulate the quantal release of GABA.

** In this context, it should be noted that presence of high levels of A_{2A} receptors is demonstrated on the terminals of the striatal axon collaterals^{23,43)} but not on the GABAergic interneurons,⁴¹⁾ favoring the A_{2A} receptor-regulation by the recurrent feedback inhibition via the axon collaterals.

mediated by A_{2A} receptors in GP might be exerted on the striatopallidal axon terminals.

These findings provide a new insight into neuromodulatory functions of adenosine in the basal ganglia. First, the striatal spiny projection neurons might receive A_{2A} receptor-mediated control in two different modes as follows. 1) Control of their neuronal activities via the striatal A_{2A} receptor on the GABAergic axon terminals with the feedback/feed-forward mechanism. Previous experiments showed that a local pharmacological direct blockade of GABA_A receptors on the striatal projection neurons *in vivo* increased the firing ratio of these cells by more than 300%⁴⁴⁾ and results in significant activation and/or disruption of motor behavior.^{45,46)} The A_{2A} receptor-mediated control of GABAergic spiny projection neuron activity might therefore be one of the most powerful determinants of the output of the striatum. 2) Control of GABA release from their axon terminals onto the GP projection neurons, a mechanism which might be more direct control of GP neuronal activities via A_{2A} receptors in GP. Second, it is noticeable that the modulatory actions of adenosine are shown in the striatum and GP, both of which are considered to perform several critical operations that influence the basal ganglia output.^{36,37,75)}

These 'dual regulations' by A_{2A} receptor have recently been demonstrated also by *in vivo* microdialysis experiments.⁴⁸⁾ Microinjection of CGS21680 into the rat striatum actually increased GABA concentrations in the globus pallidus. This increment of GABA by striatal A_{2A} receptor activation was intercepted by tetrodotoxin locally delivered to the GP to block the propagation of action potentials to the terminals. Furthermore, intrapallidal infusion of CGS21680 increased GABA levels in the GP either in the presence or absence of tetrodotoxin.

Together, A_{2A} receptors might control the striatopallidal indirect pathway at GABAergic axon terminals both in the striatum and the GP in two different modes. This dual regulation mechanism by A_{2A} receptor should work more efficiently in terms of anatomical convergence or 'funneling' in the striatum-GP system (see Fig. 1). Adenosine, via A_{2A} receptors, may significantly contribute to control the basal ganglia system as a specific neuromodulator on the striatopallidal GABAergic projection neurons.

3. Origins of extracellular adenosine

There is little evidence that adenosine is stored in synaptic vesicles or released from nerve terminals in response to an action potential in the manner of a classical neurotransmitter. The basal concentration of adenosine in the extracellular space is between 20 and 300 nM. This seems to be sufficient to tonically activate adenosine A_{2A} receptors to a modest extent,^{13,14)} as evinced by the excitatory effects of adeno-

sine receptor antagonists both *in vitro* and *in vivo*.⁵⁾ In addition to this tonic activity of adenosine, numerous conditions cause a significant elevation of the extracellular adenosine concentration. Adenosine is released from brain tissue in response to hypoxia, ischemia, electrical stimulation, chemical depolarization, and non-pathological nerve stimulation.⁵⁾

To explain the origins of extracellular adenosine, two major possible hypotheses have emerged as follows. 1) Extracellular adenosine is formed from nucleotides, released into extracellular space and catabolized to adenosine via a series of ecto-enzymes.⁴⁸⁻⁵¹⁾ Vesicular release of ATP that is co-localized with transmitters such as ACh, norepinephrine, and 5-HT are potential sources of extracellular adenosine nucleotides.⁵²⁻⁵⁴⁾ In purified cholinergic synapses from the striatum, ecto-ATPase (EC 3.6.1.15), ecto-ADPase (EC 3.6.1.6), and ecto-5'-nucleotidase (EC 3.1.3.5) have all been identified.⁵⁵⁾ The conversion of AMP to adenosine by 5'-nucleotidase is the rate-limiting step in the extracellular conversion of nucleotides to adenosine in the brain.^{55,56)} In the hippocampus, the release of nucleotides into the extracellular space results in rapid dephosphorylation to adenosine (within <1 sec), and the adenosine formed then activates nearby A₁ receptors.⁵⁵⁾ 2) Adenosine formed intracellularly is released into the extracellular space by a nonsynaptic mechanism. Intracellular formation of adenosine is directly related to cellular energetics and so is oxygen-dependent. Thus, hypoxia greatly increases adenosine production and its consequent release into the extracellular space via specific transporters, but only when energy metabolism is severely compromised.⁵⁾ Despite detailed investigations into the effects of adenosine and adenosine receptors, it is still not clear, in most cases, which cells are capable of releasing adenosine to affect a specific neuronal activity.

There may be two possible origins of extracellular adenosine that actually activate striatal A_{2A} receptors. One is ATP released from striatal acetylcholine interneuron terminals.^{52,54,55)} When ATP is released at the neuromuscular junction, it is degraded into adenosine with ecto-enzymes on the interneurons. Corticostriatal glutamatergic input into striatal GABAergic projection neurons (Fig. 1) is the other possible driving force for increasing extracellular adenosine,^{57,58)} which controls striatal GABAergic synaptic transmission⁵⁹⁾ and NMDA receptor channel conductance,^{58,60)} as follows.

The main synaptic driving forces of striatal spiny neurons include AMPA/kinate, NMDA, and GABA_A responses. Although AMPA/kinate response is the main synaptic input, the generation of action potentials in striatal neurons is greatly influenced by both GABA_A and NMDA responses.⁵⁹⁾ The glutamatergic synaptic transmission onto spiny projection neurons is suppressed by adenosine via the

A₁ receptors, but not via the A_{2A} receptors.⁶¹⁾ Alternatively, A_{2A} receptor activation by the agonist CGS21680 directly inhibits NMDA-induced (but not AMPA-induced) inward current in medium spiny neurons in rat striatal slices, by inhibiting NMDA receptor channel conductance via the phospholipase C/InsP₃/calmodulin pathway.⁶²⁾ The subunit composition and/or phosphorylation state of the NMDA receptors, expressed on the dendritic spines of the spiny neurons, appears to change in ways that affect motor performance. Conversely, striatal NMDA receptor activation leads to the increased concentration of extracellular adenosine^{57,58)} that would activate adenosine A_{2A} receptors,⁶⁴⁾ which, in turn, might inhibit NMDA receptor channel conductance.

Despite the abundance of kainate receptors, no evidence for synaptic activation of these receptors after single or repetitive stimulation of glutamatergic afferent neurons has been shown so far. It has recently been demonstrated that striatal kainate receptor activation depresses GABAergic synaptic transmission.⁵⁹⁾ This depression by kainate receptors appears to be indirect: activation of kainate receptors leads to the increase of extracellular concentration of adenosine that would act on A_{2A} receptors to depress GABAergic transmission³⁸⁾ in a retrograde way. The increase of extracellular adenosine concentration is induced by NMDA and kainate receptor activation, but not by AMPA receptor activation. Increased endogenous adenosine apparently originated from release of adenosine as well as from release and extracellular breakdown of a nucleotide.⁵⁸⁾

Together, corticostriatal glutamatergic input would activate NMDA and kainate receptors on spiny projection neurons, leading to increase extracellular concentration of adenosine, which, in turn, inhibit postsynaptic NMDA receptor channel conductance and presynaptic GABAergic transmission to facilitate the output of the striatopallidal projection neurons via A_{2A} receptors.

4. Influence of adenosine A_{2A} receptors on the actions of neurotransmitters and other neuromodulators

In addition to its direct presynaptic actions on striatopallidal neurons, A_{2A} receptor influences the action of neurotransmitter and other neuromodulators, in a way priming, triggering, or inhibiting. Such influences have been observed in intramembrane receptor-receptor interaction, cross-talking in intracellular signaling, cell-to-cell interaction, neuronal network communication, and behavioral response in a manner of 'fine tuning'.⁶⁵⁾

4.1. Influence on striatal dopaminergic activity

Dopamine D₂ receptors are coexpressed with A_{2A} receptors in the striatopallidal neurons. A_{2A}-D₂ recep-

tor interactions have been shown both within plasma membrane and intracellularly.^{66,67)} Thus A_{2A} receptor stimulation affects the affinity of dopamine for D₂ receptors, counteracts a D₂ receptor-mediated Ca²⁺ influx in A_{2A}/D₂ cotransfected cells, and abolishes the D₂ receptor-mediated tonic inhibition of c-fos expression. A number of other studies showed that adenosine A_{2A} receptors had the opposite effect on D₂ receptor mediated-effects including signal transduction, gene expression, neurotransmitter release, and behavioral responses. Hence, it has been proposed that the A_{2A}-D₂ intramembrane receptor-receptor interaction, and a downstream interaction at the level of intracellular signal transduction, may provide the main molecular and cellular mechanism underlying many of the observed effects of adenosine agonists and antagonists.^{66,67)} However, there is increasing evidence that A_{2A} receptors can operate independently of D₂ receptors. As described earlier, presynaptic A_{2A} receptors specifically modulate GABAergic synaptic neurotransmission both in the striatum and in the pallidum,^{38,39)} and inhibit GABA release from striatal synaptosomes in the absence of dopamine.⁶⁸⁾ A_{2A} receptor functions in the absence of D₂ receptors have recently been demonstrated, using D₂ receptor knockout mice (D2R-/-).^{69,70)} The D2R-/- mice have a locomotor phenotype with analogies to Parkinson's disease and alter significantly in the levels of neuropeptide genes expressed in the striatal spiny projection neurons.⁶⁹⁾ No difference in the distribution and level of expression of A_{2A} receptor mRNA and the binding properties of the receptor were found between D2R-/- and wild type mice, indicating that D₂ receptor absence had no influence on A_{2A} receptor properties. Blockade of A_{2A} receptors by an A_{2A} selective antagonist reestablishes their locomotor activity and coordination of movement and lowers the levels of striatal enkephalin expression to those in normal mice.⁷⁰⁾ These results indicate that A_{2A} and D₂ receptors have antagonistic but independent activities in controlling neuronal and motor function in the basal ganglia.

4.2. Influence on striatal cholinergic activity

In the striatum, ACh is released predominantly from relatively large (20–50 μm) aspiny interneurons. Cholinergic interneurons account for only 1–2% of the total neuronal population of the striatum, while the axonal fields of these cells are very extensive and possess much more widespread dendritic trees than do the projection neurons.⁷¹⁾ The physiological role of ACh interneurons in striatal activity still remains ambiguous. The effects of ACh are primarily mediated by activation of M₁ and M₂ muscarinic receptor subtypes.^{24,71)} High levels of the muscarinic receptor subtypes are localized differentially in several neuronal subtypes of the striatum. Striatal spiny projection neurons represent the main synaptic target of

cholinergic interneurons. Muscarinic agonists induce membrane depolarization and reduce Ca²⁺-currents in the spiny neurons. ACh regulates its own release via the activation on muscarinic autoreceptors. ACh might also act on nerve terminals of corticostriatal glutamatergic and GABAergic neurons, regulating the release of glutamate and GABA in the striatum via a presynaptic mechanism. It has been suggested that striatal ACh serves to maintain both excitable and quiescent states of striatal neurons.²⁴⁾

The A_{2A} receptors stimulate the release of ACh from striatal synaptosome preparations in process mediated by both protein kinases A and C.⁷²⁾ A similar effect is observed *in vivo*,⁷³⁾ as well as in striatal slices.⁷⁴⁾ The importance of these effects on the output pathways that is mediated through the cholinergic interneurons remains to be assessed further. Although the majority of attempts to detect A_{2A} receptor mRNA expression in striatal cholinergic neurons failed, a highly sensitive single cell RT-PCR method

as well as an *in situ* hybridization study has recently shown that both A₁ and A_{2A} receptors are expressed in striatal cholinergic neurons.²¹⁾

5. Adenosine A_{2A} receptor and Parkinson's disease

5.1. Pathophysiology of Parkinson's disease

Parkinson's disease is an age-related neurodegenerative disorder. The cardinal features of PD are resting tremor, rigidity, bradykinesia (or slowness), and gait disturbance. The primary pathology of Parkinson's disease is the degeneration of dopaminergic neurons in substantia nigra pars compacta (SNc), leading to a marked fall in striatal dopamine content which is held responsible for the onset of motor symptoms.⁷⁵⁾ Such dopamine depletion triggers a cascade of functional changes affecting the whole basal ganglia network. The most relevant alteration is hyperactive in the output nuclei of the cir-

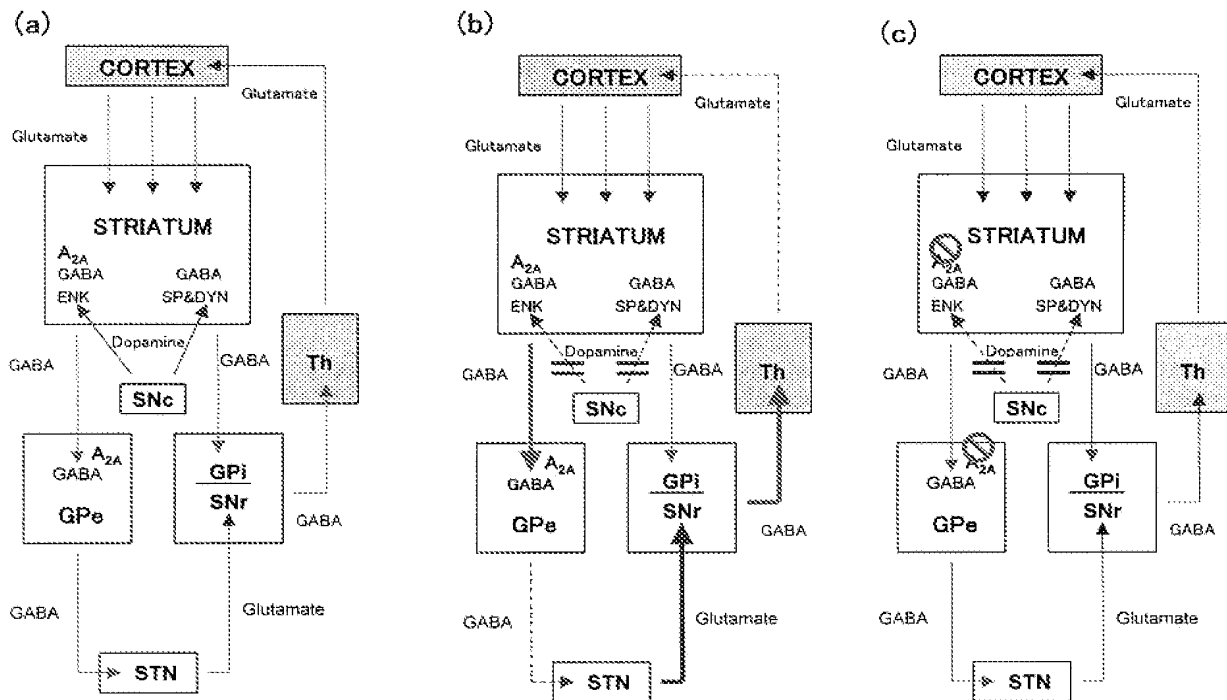


Fig. 2. Schematic Diagram for a Proposed Mechanism of anti-Parkinsonian Activity of A_{2A} Receptor Antagonists via the Regulation of Striatopallidal Neuronal Activity.

(a) Normal condition (b) Parkinson's disease, and (c) Treatment with A_{2A} antagonists in Parkinson's disease.

The schematic flow is based on Fig. 1. (a) In the normal condition, inhibitory input from the striatonigral direct pathway and disinhibitory input along the striatopallidal indirect pathway is well balanced. At striatal level, dopamine appears to facilitate transmission along the direct pathway and inhibit transmission along the indirect pathway, these two opposite effects being mediated by dopamine D₁ and D₂ receptors, respectively. Adenosine provides facilitatory control over the indirect pathway via adenosine A_{2A} receptors in the striatum and GP by the dual regulation mechanism. (b) Degeneration of nigro-striatal dopaminergic neurons depletes striatal dopamine in Parkinsonian state. The loss of striatal dopamine is thought to particularly cause a disinhibition of the striatal spiny projection neurons at the origin of the indirect pathway, which leads to a marked suppressed activity of the GPe, followed by a disinhibition of STN. The resulting imbalance between the activity in the direct and indirect pathways leads to the alterations in the GPi/SNr. Bradykinesia and akinesia observed in Parkinson's disease is postulated to result from increased GABAergic inhibition of thalamic neurons, owing to excessive excitatory drive from the STN to the GPi/SNr. (c) A_{2A} antagonists block the strong inhibitory influence of adenosine on the GPi/SNr, resulting in recovery of GPi/SNr activity. This causes relief of excessive excitatory drive from the STN to the GPi/SNr, thereby normalizing the balance between the direct and indirect pathways.

cuit, the GPe/SNr (Fig. 2-(b)). Such hyperactivity is attributed to an imbalance between the direct striatonigral pathway and the indirect striatopallidal pathway. Following destruction of the nigro-striatal dopaminergic pathway, in either Parkinson's disease or MPTP treatment of primates, altered activity of the indirect striatopallidal pathway develops. Current models thus emphasize increases in the overall activity in the striatopallidal indirect pathway (Fig. 2-(b)).^{37,76,77)} The loss of striatal dopamine leads to a reduction of tonic neuronal activity in GPe, disinhibition of the STN and then to excessive GPe/SNr drive. This results in decreased facilitation of cortical motor areas and subsequent development of akinesia and bradykinesia.

Of particular relevance is the finding that extracellular levels of adenosine (and its metabolite inosine) are higher in the striatum of MPTP-treated monkeys than in normal control monkeys.⁷⁸⁾

5.2. Proposed action mechanism of A_{2A} receptor antagonists

A_{2A} receptor antagonists block the dual control of the GABAergic neurotransmission via A_{2A} receptor in striatum and GP, leading to suppression of the excessive activation in the indirect pathway, which is caused by denervation of the nigro-striatal dopaminergic system. This might shift the striatopallidal/striatonigral neuronal imbalance towards the normal state (Fig. 2-(c)), resulting in recovery of the motor function.

Indeed, following the unilateral lesion of the nigro-striatal pathway, basal levels of extracellular GABA were increased in the ipsilateral striatum and GP.⁴⁷⁾ KW-6002, at a similar dose range to potentiate the rotation induced by a dopamine agonist,²⁷⁾ caused marked and sustained decrease of extracellular GABA levels in the GP of the lesioned rats.⁴⁷⁾ The 6-hydroxydopamine-destruction increases striatal enkephalin mRNA expression, which indicates the overactivity of striatopallidal neurons, and A_{2A} receptor antagonists reverse the increase in enkephalin mRNA expression (Aoyama, *et al.*, unpublished data). The dopamine D_2 receptor selective antagonist eticlopride has an effect similar to the dopamine depletion, in that the expression of enkephalin mRNA is increased, an effect reversed by A_{2A} antagonists.²⁴⁾

These results indicate that the proposed anti-Parkinsonian mechanism of A_{2A} receptor antagonists actually works on the striatopallidal neurons. A_{2A} receptor-mediated stimulation of ACh release and the interaction with dopamine D_2 receptors in the striatopallidal neurons might contribute to the anti-Parkinsonian mechanism of A_{2A} antagonists, although there still remain further studies for their contribution.

6. Remark in conclusion

Adenosine is a specific neuromodulator that could control basal ganglia functions by acting on the A_{2A} receptors in the striatopallidal indirect pathway. The blockade of the striatopallidal modulatory function by A_{2A} antagonists produces anti-Parkinsonian activity.

A dopamine replacement strategy, using the dopamine precursor L-dopa, has been the major treatment of Parkinson's disease therapy, which provides a dramatic benefit to virtually all patients. However, there are major limitations of L-dopa therapy, in which, after 5–10 years, L-dopa treatment is associated with the development of a series of complications including involuntary movements or dyskinesia in vast majority of patients. A_{2A} receptor antagonists reverse motor disability without inducing dyskinesia in the MPTP-primate model. A_{2A} receptor antagonists, with their action mechanisms quite different from dopaminergic agents, provide a novel therapeutic potential for the treatment of Parkinson's disease. With these concepts, KW-6002 is now in clinical trials for idiopathic Parkinson's disease. Furthermore, while a great deal of recent studies have been focused on understanding the mechanism responsible for the motor complication, further studies on A_{2A} receptor should provide a new clue to understanding Parkinson's disease as well as the physiology and pathophysiology of basal ganglia functions.

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